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Use of a Computer to Assay Motility in Bacteria[1]

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An assay was developed to study the movement of free-swimming *Escherichia coli.* Cells were videotaped through a microscope, and the videotape images were then digitized and analyzed with a computer. Angular and linear speeds were measured for wild-type *E. coli* and for a smooth and a tumbly mutant. The average angular and linear speeds of a population were directly and inversely proportional, respectively, to the time spent tumbling. Changes in angular and linear speeds were followed during the response of wild-type *E. coli* to attractant or repellent. © 1988 Academic Press, Inc.

KEY WORDS: chemotaxis; computer imaging; motion analysis; microscopy.

Motile *Escherichia coli* alternately run and tumble. This pattern is influenced by chemical attractants and repellents: Attractants suppress tumbling, while repellents enhance tumbling (1,2). The bacteria eventually adapt as they return to their unstimulated level of tumbling though the stimulus is still present.

Several methods have been developed to assay this chemotactic response. These include counting the number of bacteria that enter a capillary containing an attractant (3,4), analyzing by eye the successive photographic images of cells generating motility tracks (2,5), observing the direction of rotation of cells tethered to glass (6,7), and following individual cells in three dimensions with a tracking microscope (1).

In addition, the motility of a population of bacteria can be analyzed by eye from a videotape recording (8,9). In this report we describe an objective method to analyze videotapes of free-swimming *E. coli.* Videotapes were digitized and analyzed with equipment from the Motion Analysis Corp. A computer was used to determine the angular and linear speeds of motile bacteria. Mutant strains that exhibit smooth or tumbly behavior were analyzed, as were wild-type bacteria responding to a temporal gradient of the attractant L-aspartate or the repellent L-leucine. This work has been presented in a preliminary form in Tübingen, Federal Republic of Germany, on June 3, 1987 (10).

Related methods have been used for motility studies of *E. coli* (11), *Halobacterium halobium* (12,13), and *Euglena gracilis* (14,15).

METHODS

Media. Tryptone broth contained 1% Difco Bacto tryptone and 0.5% NaCl. Minimal medium (16) contained the carbon and energy source 25 mM sodium DL-lactate and was supplemented with the amino acids L-histidine, L-leucine, L-methionine, and L-threonine, each at 1 mM. Chemotaxis me-

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dium contained 10 mM potassium phosphate (pH 7.0), 0.1 mM potassium EDTA, and 0.1 mM L-methionine (required for tumbling in methionine auxotrophs (4)).

Strains used. All strains were derivatives of *E. coli* K12. Chemotactically wild-type strains used were AW405 (17), RP487 (18), and AW607 (19). The smooth mutant used was RP4080 containing cheR217 (20). The tumbly mutants used were AW677 containing cheB287 (21) and AW662 containing cheE618 (22), now known to be a mutation in tsr (J. S. Parkinson, personal communication).

Growth and preparation of bacteria. Bacteria were grown overnight at 35°C with rotary shaking (200 rev min^{-1}) in tryptone broth or minimal medium. For daily use such a culture was regrown in that medium to an optical density ($OD_{590\,\text{nm}}$) of 0.4 to 0.6.

For observation, cells were resuspended to an $OD_{590\,\text{nm}}$ of 0.1 either by washing them into chemotaxis medium or by diluting them into the medium in which they had grown. To do the latter, a fraction of the culture was filtered to remove bacteria, then a second fraction containing bacteria was diluted with the filtered medium.

Microscopy. A 10-μl suspension of bacteria at an $OD_{590\,\text{nm}}$ of 0.1 was placed on a glass slide, and two coverslips were placed on opposite sides of this drop. A third coverslip was then placed on the drop; this coverslip was supported by the other two coverslips.

Cells were videotaped through a phase-contrast microscope with focus maintained at the glass slide–liquid interface. An objective of 40× (numerical aperture 0.75, Zeiss 5183412) was used in conjunction with a barlow lens (Zeiss 4375749) set to 1.6×. A heat reflection filter (Zeiss 467832) was used to reduce the effect of any temperature changes caused by the microscope lamp. A video camera (MTI series 68) was separated from the main body of the microscope by a spacing tube (Zeiss 477901). The image from the camera was passed to a time–date generator (Panasonic WJ-810), followed by a contrast enhancer (Archer 15-1272). The image was then recorded at 30 frames per second by a video cassette recorder (Panasonic NV-8950). Microscopy was done on a vibration-damped platform (consisting of a marble slab supported by six No. 9 rubber plugs).

Response curves. E. coli strain AW607 was grown to an $OD_{590\,\text{nm}}$ of 0.5 in minimal medium lacking L-leucine. A fraction of this culture was filtered to remove the cells, and the remaining cells were resuspended in filtered medium to an $OD_{590\,\text{nm}}$ of 0.15. L-Aspartate or L-leucine (both from ICN Pharmaceuticals) was dissolved in filtered medium, and a 3.3-μl drop was placed on a coverslip. Cells were stimulated when the drop on the coverslip was placed in contact with a 6.6 μl drop on the slide. To control for dilution artifacts, a 3.3-μl drop of only the filtered medium was used. These experiments were done at 28°C in a constant-temperature room. (All the other experiments reported in this paper were carried out at room temperature.)

Computer analysis. Videotapes were played back on a video cassette recorder (Panasonic AG-1950) and digitized in real-time at 15 frames per second with a video processor (VP-110, Motion Analysis Corp.). The video processor detected areas of high contrast, in this case the edges of dark bacterial cells on a light background. These areas were recorded on a grid of 240 vertical by 256 horizontal pixels (2.3 pixels μm^{-1}). The *X* and *Y* coordinates of the pixels representing the outlines of cells were sent to a microcomputer (Multitech 900 PC\AT-compatible) for analysis.

Analysis was done using modular software (ExpertVision) from the Motion Analysis Corp. As shown in Fig. 1, the center of area of each cell outline (cell-center, or centroid) was calculated. Cell-centers in successive frames were then connected into paths representing the two-dimensional movement of individual cells in time (23). Each path contained the *X* and *Y* positions of the cell-center every 1/15 of a second.

The angular speed, in degrees per frame,

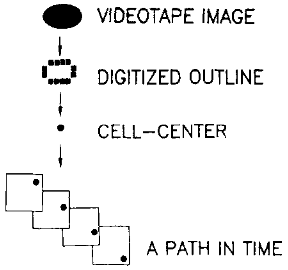

FIG. 1. Reconstruction of a cell path. For each videotape frame, an outline was generated from the image of a cell. The center of area of the outline (cell-center) was calculated, then the cell centers in consecutive frames were linked in time.

was defined as shown in Fig. 2. It was the angle subtending the direction of movement from cell-center $n - 1$ to cell-center n to the direction of movement from cell-center n to cell-center $n + 1$, divided by the time taken to change to this new direction. The linear speed, in micrometers per second, was defined as the distance between consecutive cell-centers in a path divided by the time taken to travel this distance. The equations used to calculate angular and linear speeds are given in the appendix.

Paths of non-motile and stuck cells which had an average linear speed below 5 μm s^{-1} were eliminated from further analysis. Paths less than one-third of one second long were also eliminated because they were too transient for accurate analysis.

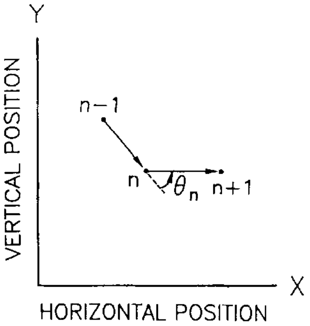

FIG. 2. Definition of angular speed. The horizontal (X) and vertical (Y) coordinates of the cell-centers were calculated from cell-outlines sampled every 1/15 of a second. The angular difference (θ_n) subtending the directions of movement between consecutive cell-centers along a path ($n - 1$, n, and $n + 1$) was calculated, and this value was divided by the time taken to change to this new direction. This defined the angular speed at cell-center n.

Visual analysis. To generate the data points shown in Fig. 4, we videotaped unstimulated RP487 (chemotactically wild type), smooth cheR and tumbly cheE and cheB mutants, and unstimulated mixtures of cheR and cheB mutants. Stimulated AW607 was also videotaped and analyzed 45 s into the response after addition of 3×10^{-7} M L-aspartate and 7 and 100 s into the response after addition of 3×10^{-2} M L-leucine (see Response curves under Methods and Fig. 5). Each tape was viewed frame by frame to score tumbles, which were identified by a characteristic jerking motion coupled with no net displacement for at least two frames. Five seconds of videotape was analyzed for each condition. Every cell in the field of view was followed for this entire observation time or until it moved out of focus, whichever came first. Tumbliness was defined as the total time spent tumbling by all the cells divided by the sum of all the cells' path durations.

RESULTS

Computer-generated paths of free-swimming bacteria. The movements of motile *E. coli* were recorded via video microscopy, and the videotape images were then digitized and analyzed by computer as described under Methods. Figure 3 shows typical computer-

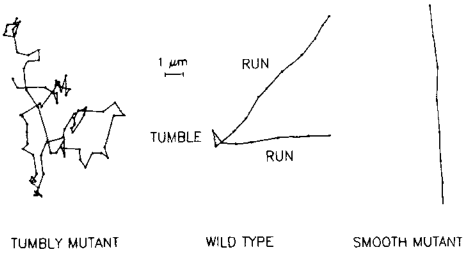

FIG. 3. Motion analysis: Computer-generated paths of bacteria. Typical paths are shown for wild type, a smooth mutant, and a tumbly mutant. Each point on a path corresponds to the position of the cell-center every 1/15 of a second.

generated paths for a wild-type bacterium and for a smooth and a tumbly mutant. The differences between these paths can be made quantitative by determining angular and linear speeds for every cell-center along each path. Angular speed is the rate of change of direction, that is, the speed of turning (Fig. 2); linear speed is the rate of movement along a straight line.

Table 1 shows the angular and linear speeds determined for wild type and a smooth and a tumbly mutant. Since large changes in direction occur during tumbles, the tumbly mutant had a higher angular speed than the wild type, and the smooth mutant had a lower angular speed. Since tumbles interrupt linear movement, the tumbly mutant had a lower linear speed than the wild type, and the smooth mutant had a higher linear speed.

Relationship of angular and linear speed to tumbliness. We wished to relate angular and linear speed to the fraction of time spent tumbling (tumbliness). To do so, we analyzed videotapes of free-swimming cells exhibiting various degrees of tumbliness. Angular and linear speed were determined by computer analysis; tumbliness was determined by eye (see Computer analysis and Visual analysis under Methods).

A comparison of tumbliness to angular speed (Fig. 4, left) revealed a direct linear relationship: A least-squares fit of this relationship gave the equation tumbliness = $1.66\,\bar{\omega} - 24.6$, where tumbliness is in percentage units and $\bar{\omega}$, the average angular speed, is in degrees per frame. Noise in the region below 40° frame^{-1} may have occurred because much of the angular speed in this region may be due to factors other than tumbling. These factors would include imaging errors incurred during digitization (24), cell wobble, and circularity in the paths of cells moving along the surface of the slide. Above 80° frame^{-1} the relationship between tumbliness and angular speed appeared to saturate.

Tumbliness was inversely linearly proportional to linear speed (Fig. 4, right). A least-squares fit of the relationship gave the equation Tumbliness = $-7.15\bar{v} + 157$, where tumbliness is in percentage units and $\bar{v}$, the average linear speed, is in micrometers per second. The noise apparent at high levels of tumbliness (below 10 $\mu\text{m s}^{-1}$) may have resulted from variations in the rates of linear movement of very tumbly cells.

To determine the error of the assay, a 10-s videotape segment of a wild-type population of RP487 suspended in tryptone broth was digitized 10 times. Subsequent analyses

TABLE 1

ANGULAR AND LINEAR SPEEDS OF FREE-SWIMMING *Escherichia coli*

	Medium					
	Tryptone broth		Minimal medium		Chemotaxis medium	
Strain	Angular speed (°frame^{-1})	Linear speed ($\mu\text{m s}^{-1}$)	Angular speed (°frame^{-1})	Linear speed ($\mu\text{m s}^{-1}$)	Angular speed (°frame^{-1})	Linear speed ($\mu\text{m s}^{-1}$)
Smooth mutant						
cheR217	22	22	27	21	27	22
Wild type						
AW405	51	16	42	17	42	17
Tumbly mutant						
cheB287	77	8	93	8	76	9

Note. Each strain was analyzed once in each medium.

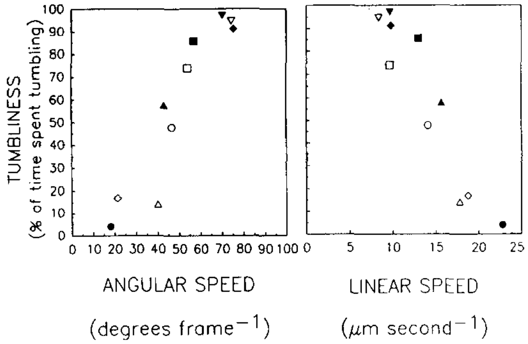

FIG. 4. Comparison of computer and visual analyses of tumbliness: Tumbliness of cells was measured by eye. Angular and linear speed were then measured by computer. Each data point represents the average of ten computer analyses. Δ, Unstimulated RP487 (chemotactically wild type) in filtered tryptone-broth; ◇, AW607 (chemotactically wild type) in filtered minimal medium responding to attractant; ♦, ○, AW607 in filtered minimal medium responding to repellent; ●, RP4080 (cheR mutant) in filtered tryptone-broth; ∇, AW677 (cheB mutant) in filtered tryptone-broth; ▼, AW677 in chemotaxis medium; □, AW662 (cheE mutant) in filtered tryptone-broth; ■, ▲, mixtures of RP4080 and AW677 in filtered tryptone-broth (see Methods).

showed the mean of the average angular speed to be 40° frame^{-1} with the standard deviation of the mean at 6.5° frame^{-1}. The mean of the average linear speed was 17 μm s^{-1} with the standard deviation of the mean at 1.1 μm s^{-1}.

Response curves. A response curve describes the change in tumbliness as a function of time upon addition of a stimulus. The change in tumbliness can be detected by analyzing changes in angular and linear speeds.

Figure 5a shows the response after addition of the attractant L-aspartate at 3 × 10^{-7} M. Angular speed decreased and linear speed increased, which indicated a decrease in tumbliness; the angular and linear speeds then returned to the unstimulated levels as the cells adapted to the presence of the L-aspartate. The response time was about 100 s. The duration of the response was related to the stimulus concentration: When cells were presented with 3 × 10^{-2} M L-aspartate, the response was complete by 450 s (data not shown).

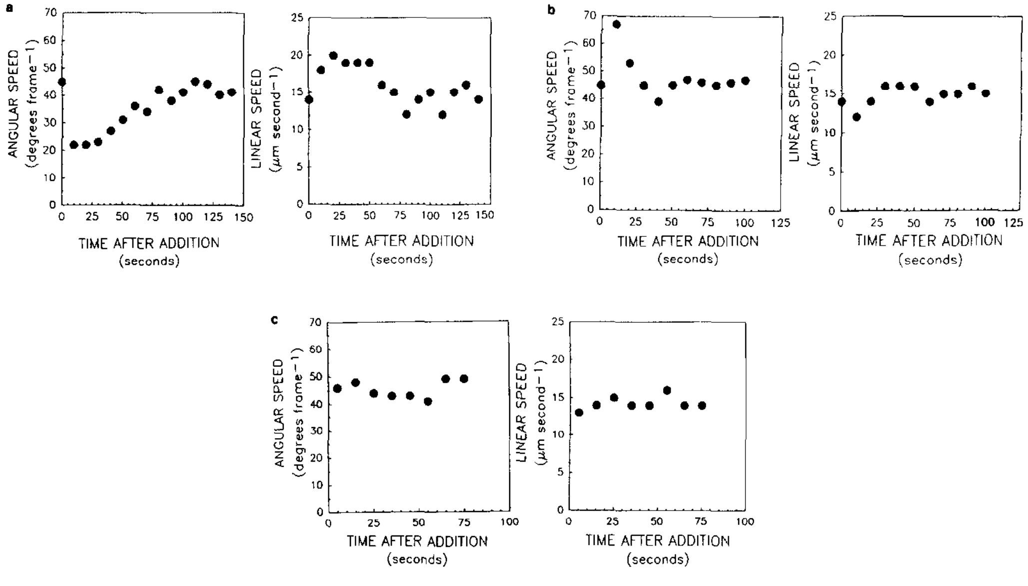

FIG. 5. Response curves for *E. coli* strain AW607: (a) Addition of 3 × 10^{-7} M of the attractant L-aspartate in filtered minimal medium. (b) Addition of 3 × 10^{-2} M of the repellent L-leucine in filtered minimal medium. (c) Addition of filtered minimal medium. In all cases: left, angular speed as a function of time; right, linear speed as a function of time. Each point represents the average over a 10-s time period and is plotted at the midpoint of this period. The first 5 to 10 s of the response curves are missing due to the time it took to add attractant or repellent and then swing the microscope objective back into place (see Methods). For a and b, the datum point at the zero-time is taken from the average of the points in c.

Figure 5b shows the response after addition of the repellent L-leucine at 3×10^{-2} M. Angular speed increased and linear speed decreased, which indicated an increase in tumbliness. Eventually the cells adapted, slightly overshooting their ultimate recovery value of angular speed. The response time was about 50 s.

Figure 5c shows a control that failed to respond to dilution with the filtered medium to which attractant and repellent had been added in Figs. 5a and 5b, respectively. This represented the baseline (prestimulus) level of angular and linear speed.

DISCUSSION

We have described a motion-analysis assay capable of objectively measuring the motility of *E. coli.* The procedure is useful to characterize the unstimulated behavior of wild-type, smooth, and tumbly strains of *E. coli* (Table 1 and Fig. 4) and to quantify stimulus–response curves (Fig. 5). We recommend using both angular and linear speed to measure the effects of stimuli and to determine response times.

An advantage of this assay is the statistical significance of a response curve: We typically have 15 motile cells per frame and digitize at 15 frames per second, so each 10-s point in Fig. 5 represents the movement of over 2000 cell-frames. A disadvantage is that runs and tumbles are not evaluated individually, so information about individual cells is not available.

Behavioral and biochemical studies could be conducted simultaneously, so this methodology may be useful in the determination of the biochemical basis of behavior in *E. coli.* The assay should be generally applicable to other types of taxes in *E. coli,* as well as to motility studies in other organisms.

APPENDIX

From the X, Y and time values of individual cell-centers (see Computer analysis under Methods), angular and linear speeds were calculated. For the nth cell-center in a path, angular speed (ω), in degrees per frame, was defined as

$$\omega_n = \left| \frac{\arctan\left[\dfrac{(Y_{n+1} - Y_n)}{(X_{n+1} - X_n)}\right] - \arctan\left[\dfrac{(Y_n - Y_{n-1})}{(X_n - X_{n-1})}\right]}{(1/15)} \right|$$

For the nth cell-center in a path, linear speed (ν), in micrometers per second, was defined as

$$\nu_n = \frac{[(X_n - X_{n-1})^2 + (Y_n - Y_{n-1})^2]^{1/2}}{(1/15)}.$$

Information specific for the motion analysis instrumentation used in this paper is available from the authors.

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